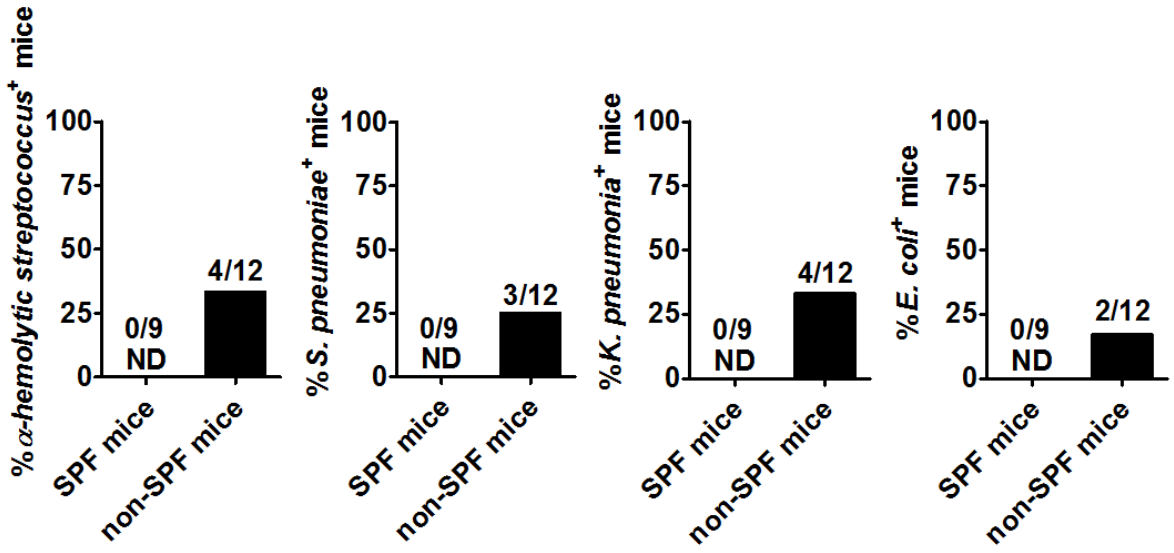
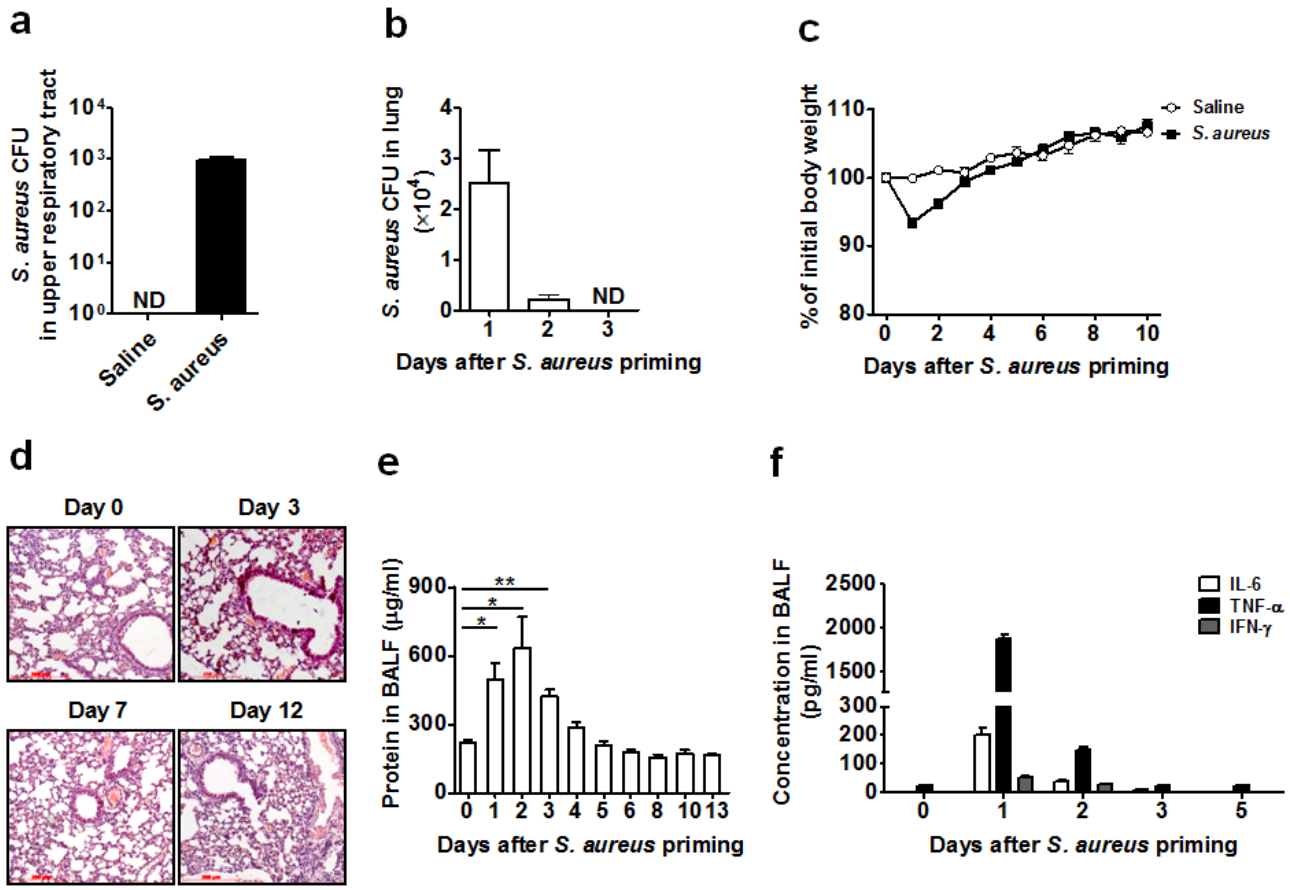
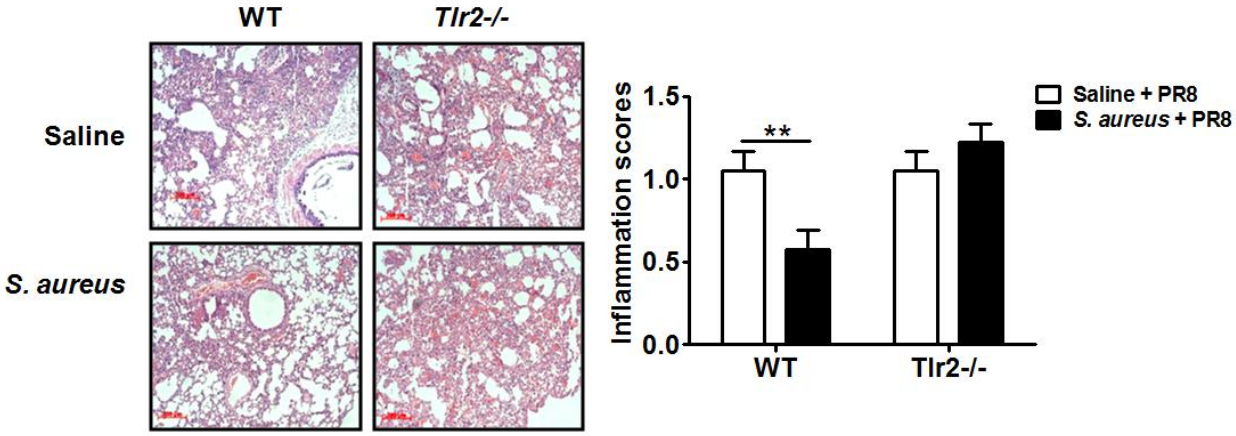


Supplementary Figure S1. Bacterial diversity in the upper respiratory tract of non-SPF mice.

Lavage fluids from the upper respiratory tract of SPF mice and non-SPF mice were cultured with blood plates. Different kinds of bacteria were analyzed 3 days later by bacterial morphological examination, and then the percentages of SPF and non-SPF mice colonized by various bacteria in the upper respiratory tract were counted.

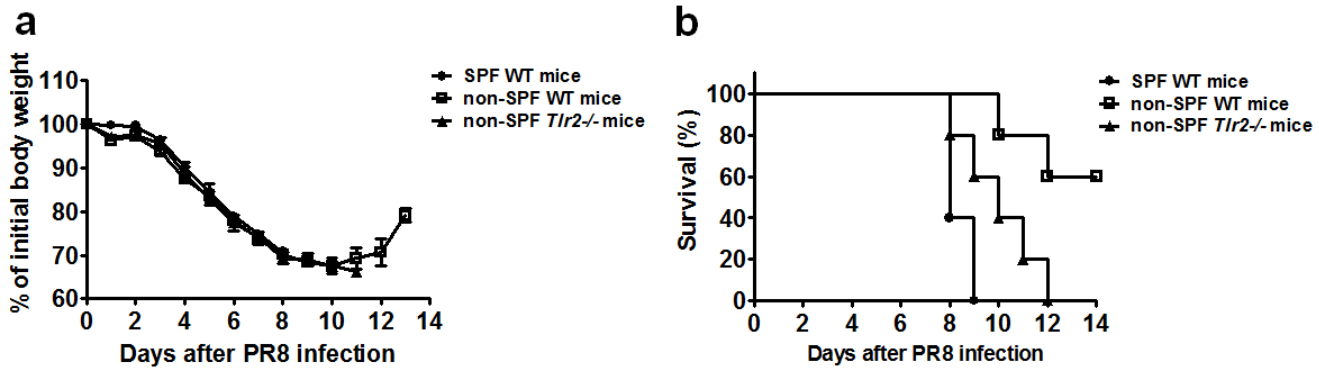


Supplementary Figure S2. *S. aureus* commensally colonizes the upper respiratory tract of mice. WT mice were i.n. primed with saline or 1×10^7 CFU of *S. aureus*. **(a)** *S. aureus* burdens in the upper respiratory tract 3 days after *S. aureus* priming. **(b)** *S. aureus* burdens in the lungs at the indicated times after *S. aureus* priming. **(c)** Change in body weight, **(d)** H&E staining of lung, **(e)** BCA protein assay of total protein and **(f)** ELISA of cytokines in BALF at the indicated times after *S. aureus* priming. ND, not detected. Two-tailed Student's *t*-tests, **P*<0.05, ***P*<0.01. Data are expressed as mean ± s.e.m. Data represent three independent experiments with at least five mice per group in **(a, b, c, e, and f)**, or represent three independent experiments with three mice per group in **(d)**.



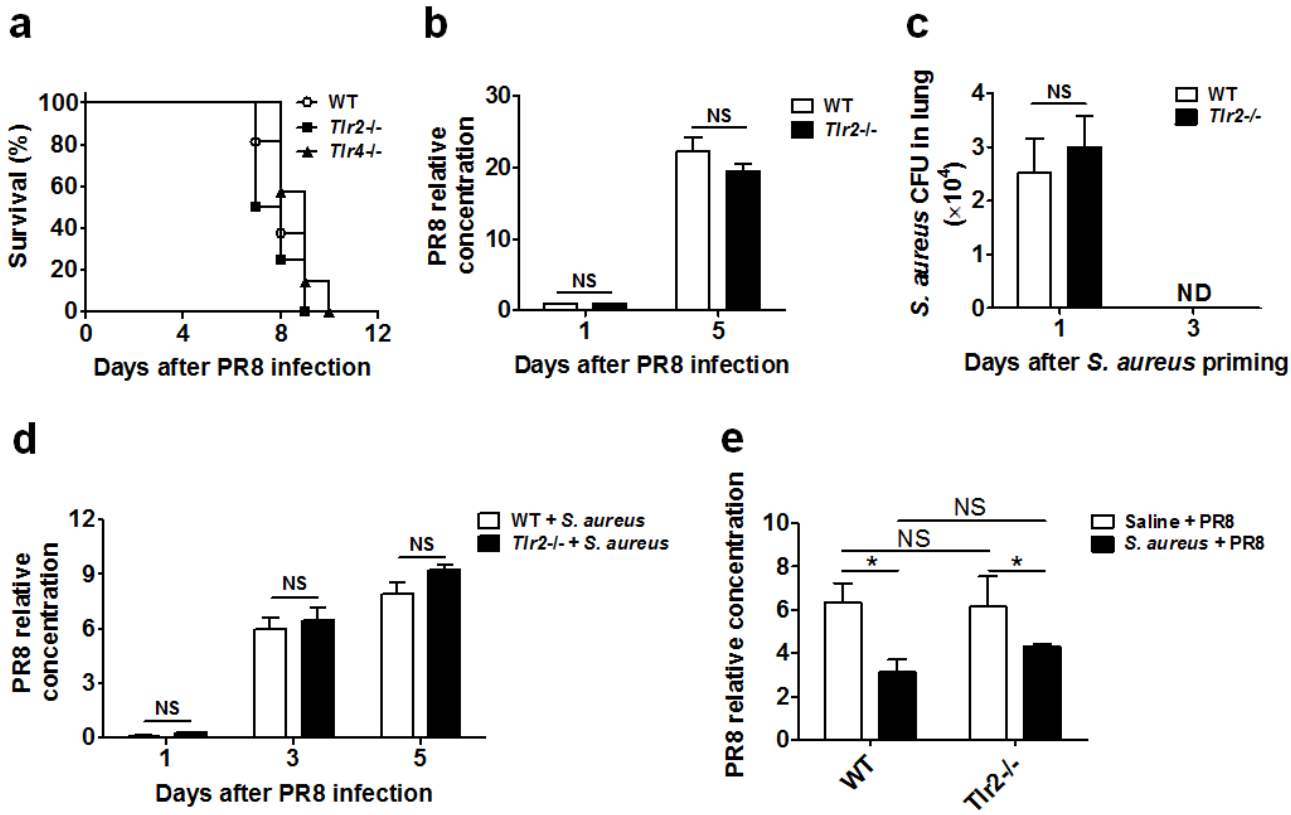
Supplementary Figure S3. *S. aureus* priming attenuates lung injury caused by a lethal dose of PR8 infection.

H&E staining and inflammation scores of lungs from control WT and *Tlr2*$^{-/-}$ mice as well as from *S. aureus*-primed WT and *Tlr2*$^{-/-}$ mice at day 5 after infecting with 0.5 HA of PR8. Two-tailed Student's *t*-tests, $^{**}P<0.01$. Data are expressed as mean ± s.e.m. Data represent three independent experiments with three mice per group.



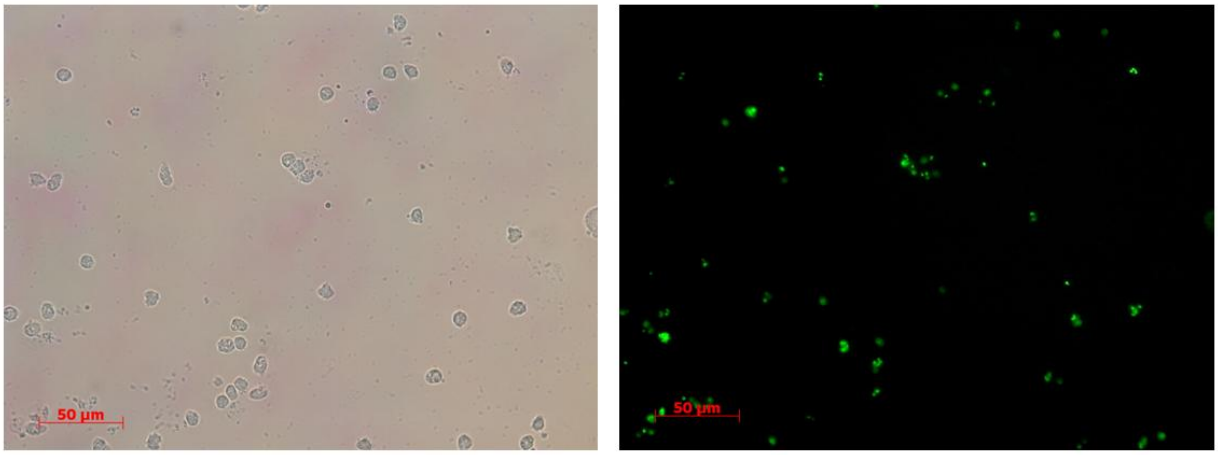
Supplementary Figure S4. Non-SPF *Tlr2*$^{-/-}$ mice cannot resist to influenza-mediated death.

(a) Change in body weight of WT mice and *Tlr2*$^{-/-}$ mice housed in a SPF or non-SPF environment at the indicated times after infecting with 0.5 HA of PR8. **(b)** Survival of SPF WT mice, non-SPF WT mice and non-SPF *Tlr2*$^{-/-}$ mice after infecting with 0.5 HA of PR8. Data are expressed as mean ± s.e.m. Data represent two independent experiments with five mice per group.



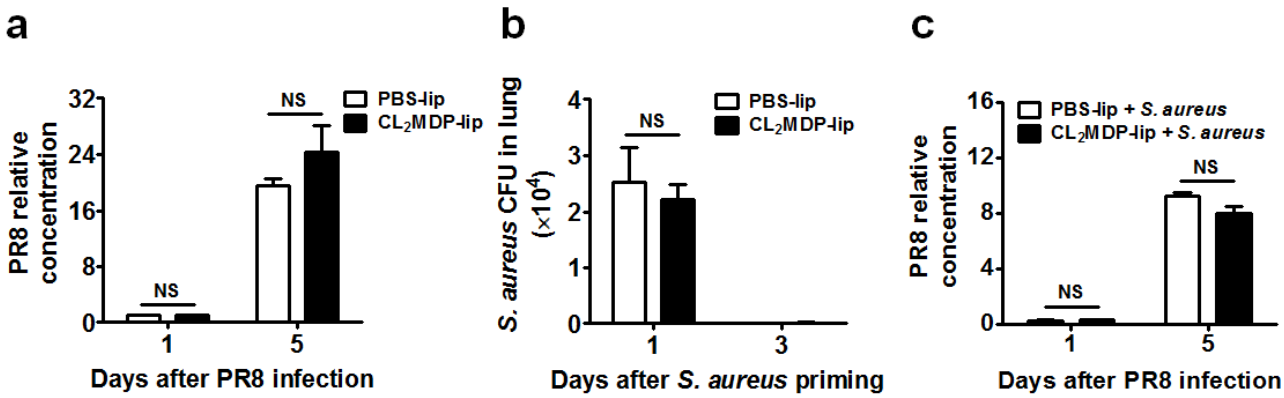
Supplementary Figure S5. TLR2 deficiency does not have a direct influence on influenza infection and *S. aureus* priming.

(a) Survival of WT, *Tlr2*$^{-/-}$ and *Tlr4*$^{-/-}$ mice after infecting with 0.5 HA of PR8. **(b)** PR8 loads in the lungs from WT and *Tlr2*$^{-/-}$ mice at days 1 and 5 after infecting with 0.1 HA of PR8. **(c)** Bacterial burdens in the lungs from WT and *Tlr2*$^{-/-}$ mice at days 1 and 3 after *S. aureus* priming. **(d)** PR8 loads in the lungs from control WT and *Tlr2*$^{-/-}$ mice as well as from *S. aureus*-primed WT and *Tlr2*$^{-/-}$ mice at day 5 after infecting with 0.5 HA of PR8. **(e)** PR8 loads in the lungs from *S. aureus* primed WT and *Tlr2*$^{-/-}$ mice at days 1, 3 and 5 after infecting with 0.1 HA of PR8. ND, not detected. Two-tailed Student's *t*-tests, NS: not significant. Data are expressed as mean ± s.e.m. Data represent three independent experiments with five mice per group in **(a)**, or represent two independent experiments with three mice per group in **(b-d)**.



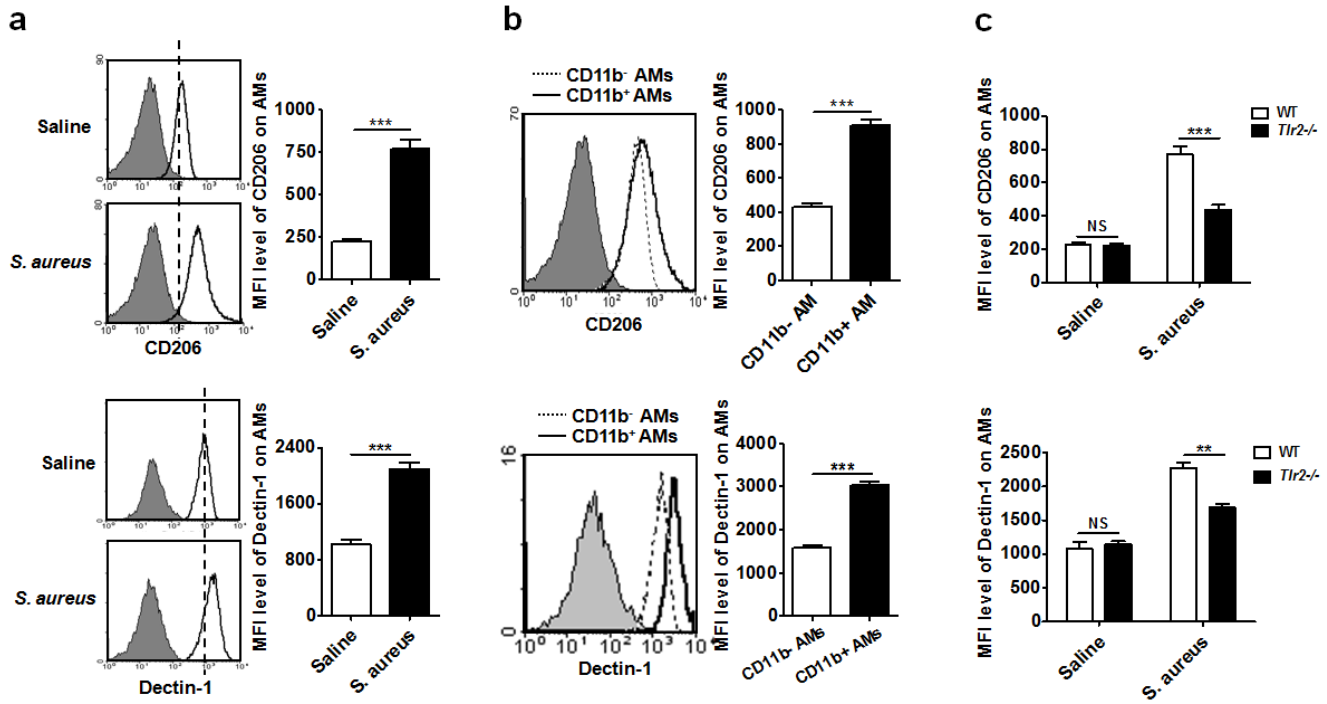
Supplementary Figure S6. AMs can directly engulf GFP-labeled *S. aureus*.

Alveolar macrophages from *S. aureus*-primed mice co-cultured with GFP-labeled *S. aureus* for 1h, and then mounted on the slides, and finally analyzed by fluorescence microscope (magnification ×400).



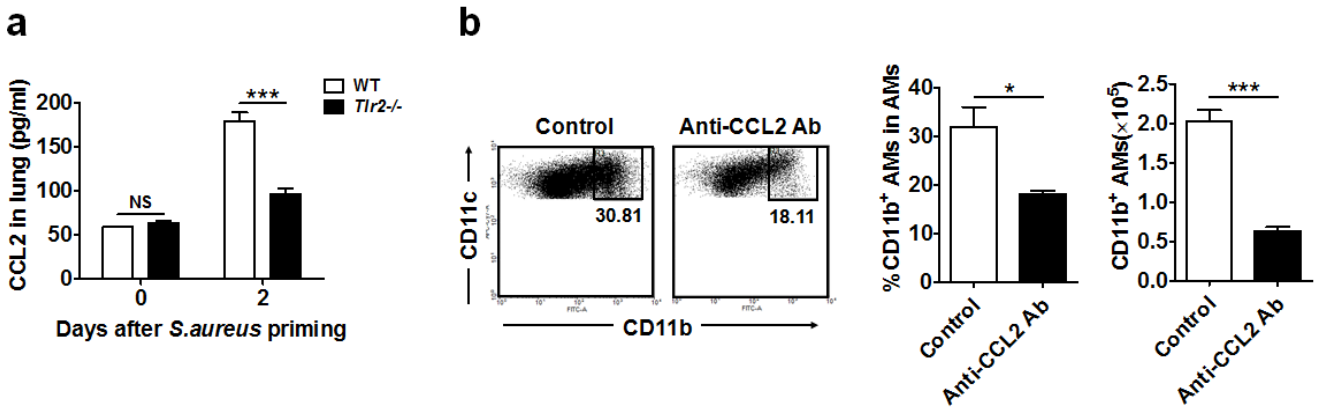
Supplementary Figure S7. AM depletion does not have a direct influence on influenza infection and *S. aureus* priming.

(a) PR8 loads in the lungs from PBS-lip-treated and CL_2MDP-lip-treated WT mice at days 1 and 5 after infecting with 0.1 HA of PR8. **(b)** Bacterial burdens in the lungs from PBS-lip-treated and CL_2MDP-lip-treated WT mice at days 1 and 3 after *S. aureus* priming. **(c)** PR8 loads in the lungs from *S. aureus* primed PBS-lip-treated and CL_2MDP-lip-treated WT mice at days 1 and 5 after infecting with 0.1 HA of PR8. Two-tailed Student's *t*-tests, NS: not significant. Data are expressed as mean ± s.e.m. Data represent three independent experiments with three mice per group.



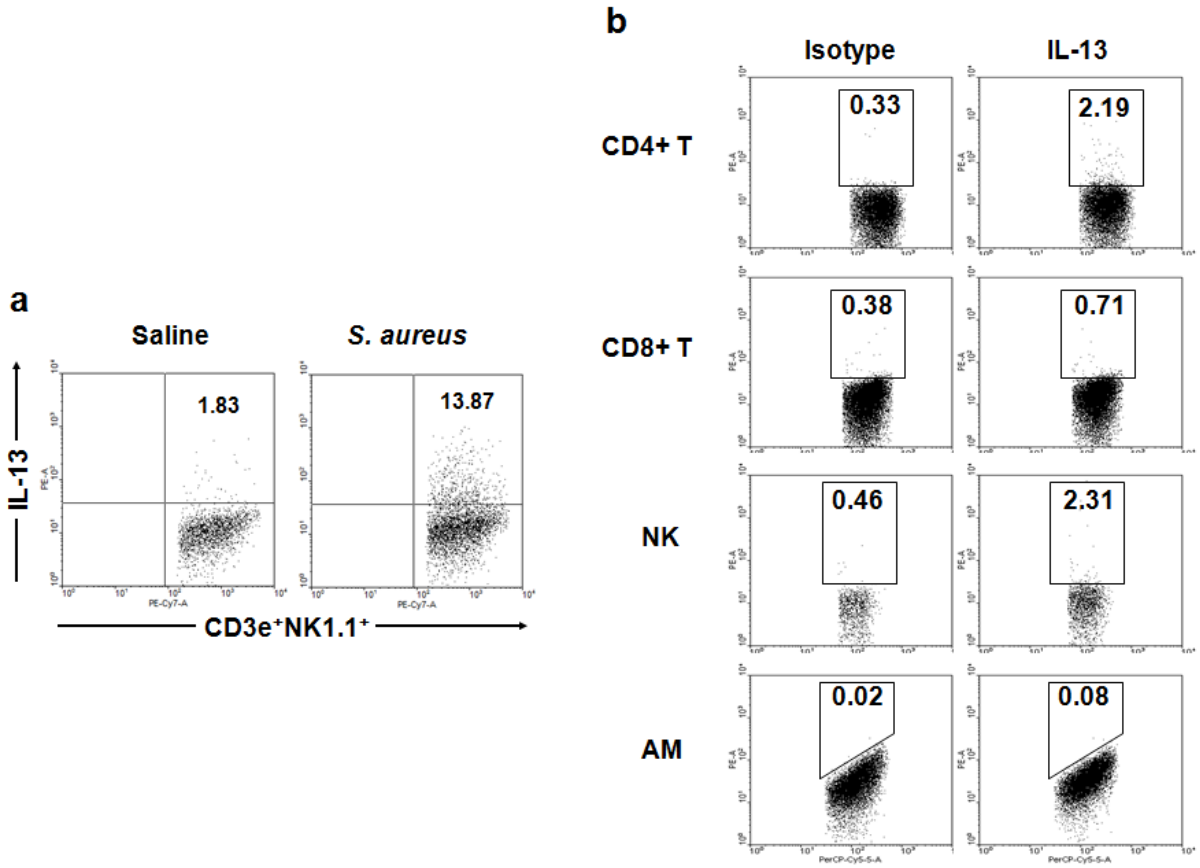
Supplementary Figure S8. *S. aureus* priming up-regulates CD206 and Dectin-1 expression on AMs.

(a) CD206 and Dectin-1 expression on AMs from WT mice at day 3 after *S. aureus* priming. **(b)** CD206 and Dectin-1 expression on $CD11b^-$ and $CD11b^+$ AMs from WT mice at day 3 after *S. aureus* priming. **(c)** WT and *Tlr2*$^{-/-}$ mice were primed with *S. aureus*. MFI levels of CD206 and Dectin-1 on AMs are shown. Two-tailed Student's *t*-tests, NS: not significant, ***P*<0.01, ****P*<0.001. Data are expressed as mean ± s.e.m. Data represent three independent experiments with three mice per group.

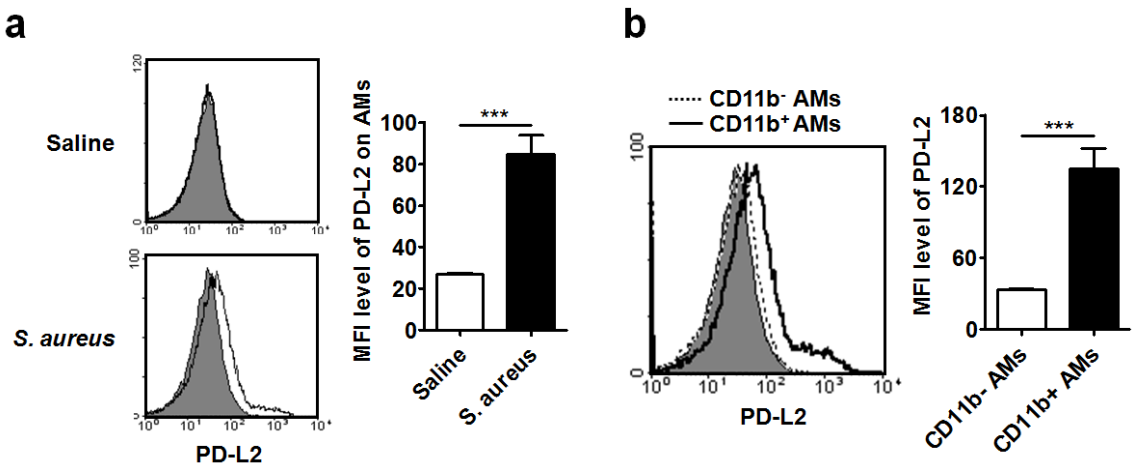


Supplementary Figure S9. *S. aureus* priming promotes CCL2 expression to recruit peripheral blood monocytes into lung.

(a) ELISA of CCL2 in the lungs from WT and *Tlr2*$^{-/-}$ mice 2 days after *S. aureus* priming. **(b)** Percentage and number of CD11b^{+} AMs from control WT mice and anti-CCL2 neutralizing antibody-treated WT mice 3 days after *S. aureus* priming. Two-tailed Student's *t*-tests, **P*<0.05, ****P*<0.001. Data are expressed as mean ± s.e.m. Data represent three independent experiments with three mice per group.

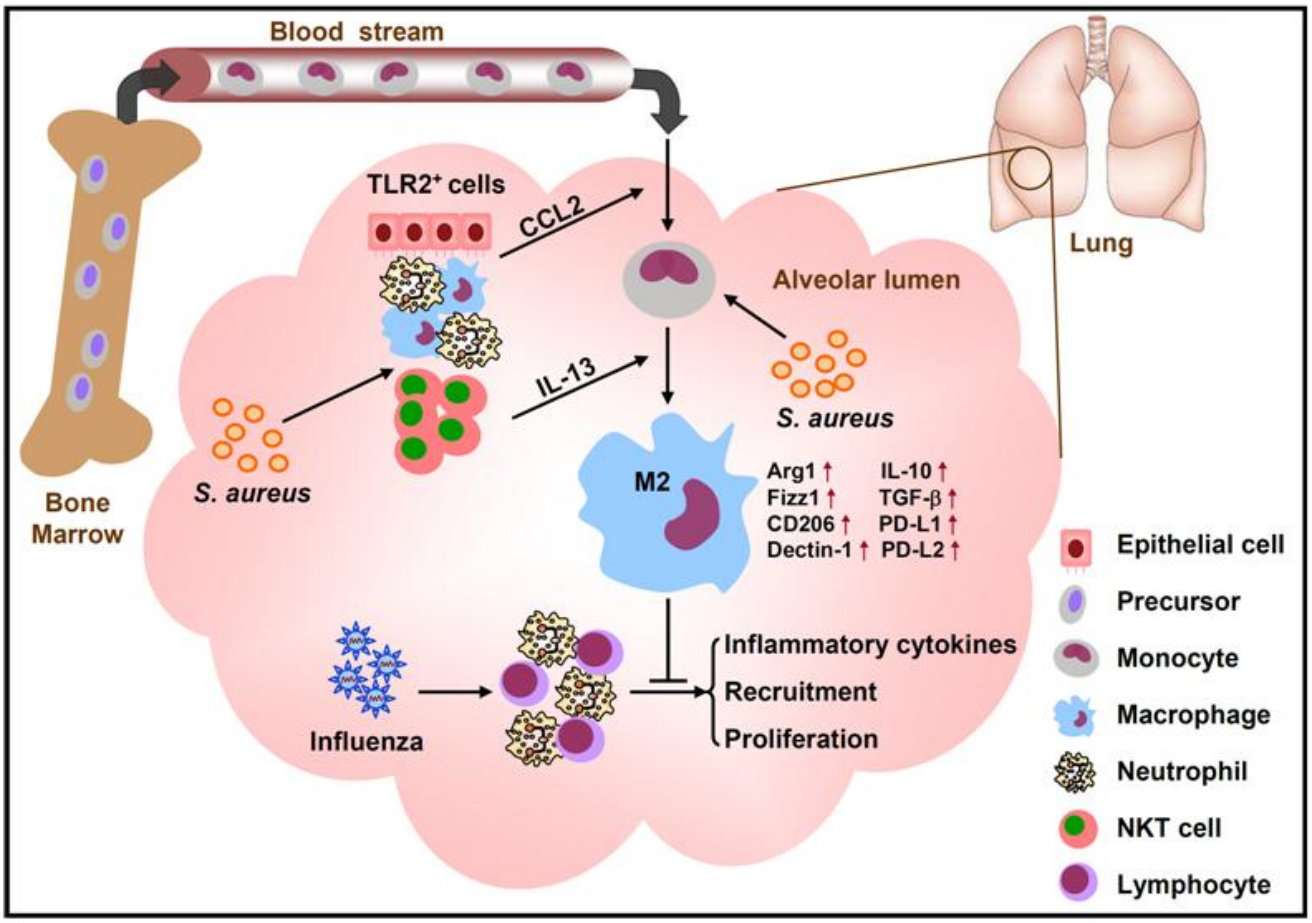


Supplementary Figure S10. *S. aureus* priming promotes high IL-13 expression by NKT cells. **(a**) IL-13 expression in NKT cells (gate $CD3^{+}NK1.1^{+}$) in BALF from WT mice at day 3 after *S. aureus* priming. **(b)** IL-13 expression in $CD4^{+}$ T, $CD8^{+}$ T, NK and AM cells in BALF from WT mice at day 3 after *S. aureus* priming. Data represent three independent experiments with three mice per group.



Supplementary Figure S11. *S. aureus* priming up-regulates PD-L2 expression on AMs.

(a) PD-L2 expression on AMs from WT mice at day 3 after *S. aureus* priming. **(b)** PD-L2 expression on CD11b^{-} and CD11b^{+} AMs from WT mice at day 3 after *S. aureus* priming. Two-tailed Student's *t*-tests, ***P<0.001. Data are expressed as mean ± s.e.m. Data represent three independent experiments with three mice per group.



Supplementary Figure S12. Working model of *S. aureus*-induced TLR2-dependent protection by M2 macrophages against influenza-induced lung inflammation.

The cellular components of the commensal bacterium *S. aureus* continually stimulate $TLR2^+$ cells, such as epithelial cells, macrophages and neutrophils, to produce chemokines and cytokines in the alveoli, which recruit peripheral $CCR2^+$ blood monocytes into the alveoli and promote NKT cells to produce IL-13. NKT cell-derived IL-13 further promotes M2 polarization of the recruited monocytes. M2 macrophages with a typical phenotype of $Arg1^+Fizz1^+CD206^{hi}Dectin\text{-}1^{hi}$ produce high levels of IL-10, TGF-β, PD-L1 and PD-L2, which are responsible for inhibiting the infiltration and expansion of inflammatory cells in the alveoli induced by influenza infection. Together, this coordinated mechanism eventually prevents mice from influenza-mediated lung injury and death.

Supplementary Table S1. Primers Used for RT-qPCR	
Influenza virus A (Matrix protein)	5’-GGACTGCAGCGTAGACGCTT-3’ (forward)
	5’-CATCCTGTTGTATATGAGGCCCAT-3’ (reverse)
β-actin	5’-TGACGTTGACATCCGTAAAGACC-3’ (forward)
	5’-CTCAGGAGGAGCAATGATCTTGA-3’ (reverse)
iNOS	5’-CTGCAGCACTTGGATCAGGAACCTG-3’ (forward)
	5’-GGGAGTAGCCTGTGTGCACCTGGAA-3’ (reverse)
Arg-1	5’-CAGAAGAATGGAAGAGTCAG-3’ (forward)
	5’-CAGATATGCAGGGAGTCACC-3’ (reverse)
Fizz1	5’-TCCCAGTGAATACTGATGAGA-3’ (forward)
	5’-CCACTCTGGATCTCCCAAGA-3’ (reverse)
Ym-1	5’-GGGCATACCTTTATCCTGAG-3’ (forward)
	5’-CCACTGAAGTCATCCATGTC-3’ (reverse)
IL-10	5’-ATGCAGGACTTTAAGGGTTACTTG-3’ (forward)
	5’-TAGACACCTTGGTCTTGGAGCTTA-3’ (reverse)
TGF-β	5’-TGACGTCACTGGAGTTGTACGG-3’ (forward)
	5’-GGTTCATGTCATGGATGGTGC-3’ (reverse)

Supplementary Table S2. Anti-mouse Monoclonal Antibodies (mAbs) Used for Flow Cytometry				
Product Name	**Clone**	**Catalog Number**	**Company**	**Isotype Control**
FITC-CD3e	145-2C11	553062	BD	Armenian Hamster IgG1, κ
PE-TLR2	6C2	12-9021	eBioscience	Rat IgG2b, κ
PE-CD11b	M1/70	553311	BD	Rat IgG2b, κ
PE-PD-L1	MIH5	558091	BD	Rat IgG2a, λ
PE-CD8a	53-6.7	553033	BD	Rat IgG2a, κ
PE-IL-13	eBio13A	12-7133	eBioscience	Rat IgG1, κ
PerCP-cy5.5- NK1.1	PK136	551114	BD	Mouse IgG2a, κ
PerCP-cy5.5-F4/80	BM8	45-4801	eBioscience	Rat IgG2a, κ
PE-cy7-NK1.1	PK136	552878	BD	Mouse IgG2a, κ
APC-PD-L2	TY25	560086	BD	Rat IgG2a, κ
Alexa Fluor® 647-Dectin-1	2A11	MCA2289A647T	AbD serotec	Rat IgG2b
Alexa Fluor® 647-CD206	MR5D3	MCA2235A647	AbD serotec	Rat IgG2a
APC-eFluor® 780-CD11c	N418	47-0114	eBioscience	Armenian Hamster IgG
Alexa Fluor® 647-Ki67	SolA15	51-5698-82	eBioscience	Rat IgG2a, κ